Provide Human Reticulocytes for in vitro Culturing of Malaria Parasites

NOTE: The Solicitations and topics listed on this site are copies from the various SBIR agency solicitations and are not necessarily the latest and most up-to-date. For this reason, you should use the agency link listed below which will take you directly to the appropriate agency server where you can read the official version of this solicitation and download the appropriate forms and rules.

The official link for this solicitation is: http://www.acq.osd.mil/osbp/sbir/solicitations/sbir113/

Agency:
Department of Defense

Release Date:
July 28, 2011
Branch:
n/a

Open Date:
July 28, 2011
Program / Phase / Year:
SBIR / Phase I / 2011

Application Due Date:
September 28, 2011

Solicitation:
2011.3

Close Date:
September 28, 2011
Topic Number:
A11-124

Description:

TECHNOLOGY AREAS: Biomedical

ACQUISITION PROGRAM: Office of the Principal Assistant for Acquisition, USAMRMC

OBJECTIVE: To provide human reticulocytes capable of being invaded by the malaria parasite Plasmodium vivax in numbers sufficient to support long term in vitro culturing of the parasite.

DESCRIPTION: Malaria is an infectious disease caused by protozoan parasites of the genus Plasmodium transmitted by Anopheles mosquitoes. While five species have been shown to infect humans, two malaria species cause the majority of disease burden. Plasmodium falciparum (P. falciparum), the most virulent of these, is associated with the majority of severe malaria and mortality. P. vivax, the second major cause of malaria worldwide and the major cause of malaria outside Africa, is associated with chronic malaria characterized by relapse after months to years of asymptomatic dormancy. P. vivax differs considerably from P. falciparum in that it invades only reticulocytes (immature red blood cells) expressing Duffy blood group surface antigens; produces mature, infective gametocytes prior to clinical symptoms; and can produce dormant liver-stage hypnozoites responsible for relapse many months after the initial infection. Robust in vitro culture methods are critically needed for basic and applied research to develop new vaccines and drugs for

malaria. As an example, many of the advances in P. falciparum research were enabled by the continuous propagation methods developed in the 1970's.

The greatest impediment in developing in vitro blood culture methods for P. vivax is that normal peripheral blood contains only 0.5-1.5% reticulocytes, an insufficient number for maintaining long term P. vivax cultures in vitro. The key deliverable for this SBIR will be a method of producing large numbers (log 12 to log 13) of reticulocytes on a regular basis (Monthly) suitable for use in long term cultures of P. vivax. Two characteristics of reticulocytes are critical to P. vivax invasion and propagation (competent). The Duffy blood group surface antigens must be expressed and adult hemoglobin levels must be high. Preliminary, proof of concept studies will require log9 to log10 competent reticulocytes on a by-weekly basis. In the assay development phase log10 to log11 reticulocytes monthly will be required. Finally, the active screening will require methods of providing log12 to log13 and greater competent reticulocytes on a monthly basis. In addition to the reticulocytes, all appropriate specialized media will need to be developed.

One promising approach to the production of high numbers of blood cells is to use the expansion and differentiation of Human Stem Cells (HSC). With the advent of innovative stem cell technologies, an abundant new source of reticulocytes is possible. Defense Advanced Research Projects Agency (DARPA) is currently funding projects which support the generation of large numbers of mature erythrocytes from placenta- and umbilical cord- derived stem cells. Dr. Douay, University of Paris, has developed HSC expansion and differentiation media conditions for producing large numbers of erythrocytes. (Giarratna M, Kobari L, Lapilloni H, Chalmers D, Kirer L, Cynober T, Marden M, Wajcman H and Douay L. Ex vivo Generation of Fully Mature Human Red Cells From Hematopoetic Stem Cells. Nature Biotechnology. 23: 69 2005.). The key to leveraging these technologies is to arrest erythrocyte development at the reticulocyte stage.

Although there are no continuous in vitro P. vivax cultivation systems, several laboratories have reported limited in vitro blood- and liver- stage cultivation [see reviews, Udomsangpetch et al Parasitology international 56(1):65-9, 2007 and Trends in Parasitology. 24: 85. 2008]. These studies demonstrate that in vitro culture of P. vivax is possible. A continual supply of reticulocytes at low cost in great numbers is the key to the development of useful culture technologies.

Finally, the ability to aggregate large numbers of reticulocytes might be an enhancement to the efforts to establish a donor-free blood supply. The differentiation of reticulocytes to mature erythrocytes is an incompletely understood process. Performing this differentiation in vitro in high efficiencies is likely to be difficult. One way to bypass this would be to transplant reticulocytes and allow them to differentiate in vivo.

PHASE I: This Phase will demonstrate the feasibility of producing log9 to log10 Duffy positive reticulocytes with adult hemoglobin greater than 50% and will identify demonstration success criteria.

PHASE II: Awardee will provide WRAIR with log9 to log10 viable, Duffy positive human reticulocytes along with any specialized procedures or media needed to maintain the cells. The % reticulocytes and % Duffy positive of each shipment will be monitored and reported. The reticulocyte fraction should be greater than 60% of the whole cell count. Greater than 50% of the reticulocytes should be Duffy positive. These cells will be tested by WRAIR to determine their ability to sustain parasite invasion.

PHASE III: The awardee will have the capacity to provide log12 to log13 Duffy positive reticulocytes with adult hemoglobin greater than 50% and with the reticulocyte count being 60% of the whole cell count or greater on a monthly basis. The cells should be capable of supporting P. vivax growth in culture. This technology will provide military and civilian laboratories the capability of screening chemical compounds in vitro for efficacy against P. vivax malaria parasites. Culture capability is the first step used for drug discovery efforts against P. falciparum and has been a major contributor to the fielding of all current anti-falciparum treatment and prophylactic drugs. Efforts to develop anti malarial vaccines are also highly dependent on culturing capability. This technology would open P. vivax vaccine development to government and civilian laboratories. Finally the technology developed here can also be applied to blood farming/blood banking activities. The availability of

producing large numbers of reticulocytes with specific characteristics would be useful for transfusion purposes in a clinical setting.